Effects of a Copper-Iodine Complex Wound Irrigation Solution on the reduction of biofilms grown on implant materials and *in vivo* porcine wounds

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Introduction

Clyra's "Bioclynse®" Wound Irrigation Solution (WIS) is an FDA 510(k) cleared medical device indicated in wound management, cleansing, irrigating, moisturizing, and debriding of acute and chronic dermal lesions that are partial or full thickness wounds. These indications include 1st and 2nd degree burns, stage I–IV pressure ulcers, diabetic ulcers, stasis ulcers, abrasions and minor skin irritations, post-surgical wounds, grafted and donor sites.

The WIS Preservative in Solution is based on a powerful Copper-Iodine Complex technology that releases a tailored amount of free iodine, I_2 (up to 250 ppm). This free iodine, acting in concert with copper ions, produces a synergistic effect that helps remove contamination within the solution and assists in the cleansing, irrigating, and debriding of wounds. The mechanism of action of the WIS is based on mechanical action of the pressurized fluid coming from the dispensing container or through a pulsed lavage system, which moves across the wound surface aiding in the removal of contamination or foreign objects such as dirt, debris or microorganisms.

This unique Copper-Iodine Complex of the WIS's preservative can neutralize a broad number of pathogens such as bacteria, viruses, yeast, and fungi without evoking bacterial resistance[1-4]. The WIS has been proven to be safe, non-cytotoxic, non-pyrogenic, non-irritating, and non-sensitizing to dermal tissue[5,6]. It can be applied directly to the wound bed and can be used with static or pulsed lavage, ultrasonic debridement and negative pressure wound therapy (instillation mode). Also, importantly, it does not need to be rinsed after treatment or application in either static or dynamic mode, potentially reducing procedure times.

The purpose of this study is to quantitatively evaluate the effect of the Copper-Iodine Complex Wound Irrigation Solution on biofilm on two commonly used implant material substrates (silicone and titanium alloy), and in an *in vivo* porcine model.

Materials and Methods

Trial #1 - Silicone Trial - mature biofilms of *S. epidermidis* ATCC 35984 were grown for 48 hours on smooth silicone breast implant shell material coupons (1 cm^2) using a CDC Biofilm Reactor. The reduction of biofilm was evaluated at three exposure times (5h, 24h, and 72h). Three independent experiments were conducted, and log reduction data were plotted as mean values with standard deviations. Untreated silicone coupons with initial bacterial load of ~10^7 CFU/cm^2 acted as controls.
The testing approach used methods adapted from ASTM E3161-18, "Standard Practice for Preparing A *Pseudomonas aeruginosa* Or *Staphylococcus aureus* Biofilm Using The CDC Biofilm Reactor".
For comparison purposes, efficacy data for other commercially available wound cleansers against *S. epidermidis* were drawn from published information, where their biofilms were grown on hydroxyapatite or titanium alloy substrates. Contact times of Irrisept, Betadine, Vashe and Bactisure are determined by manufacturer's instructions and the need to rinse out.

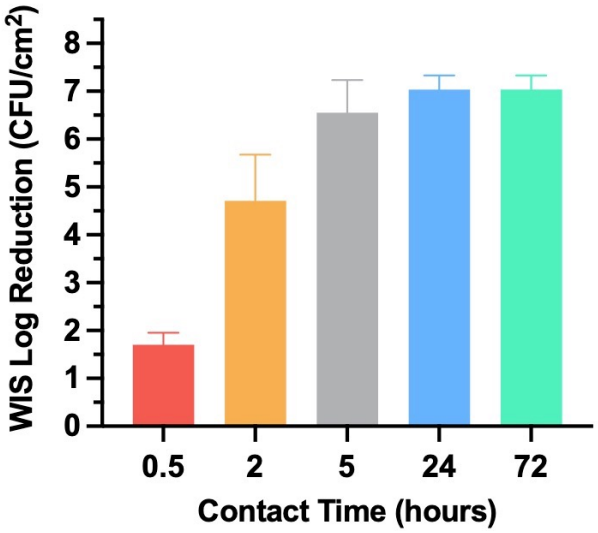
Trial #2 - Titanium Alloy Trial - mature biofilms of *Staphylococcus aureus* (MRSA) MBL strain 10943, a clinical chronic wound isolate, were grown for 72h on Grade 5 (6AL-4V) titanium coupons (18.75 cm^2) using a Drip Flow Biofilm Reactor®. Three independent experiments were conducted, each with evaluation of biofilm reduction at four exposure times (5 min, 0.5h, 2h, and 24h) as shown in Table 3.
A variation of this study was then performed using a pulsed lavage system (InterPulse® by Stryker) for 1.5 min (time required to dispense an entire 32oz WIS bottle) simulating an irrigation procedure. The coupons were irrigated at a distance of 3±1 inches with the pulsed lavage system guided over the coupon back and forth at approximately 1 sweep per second. After spraying, coupons were returned to the DFR and maintained under static immersion for 30 min and 2 hours. Untreated Grade 5 (6AL-4V) titanium coupons with initial bacterial load of 10^6-10^7 CFU/cm^2 acted as controls.
The testing approaches used methods adapted from ASTM E2647-20, "Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown Using Drip Flow Biofilm Reactor with Low Shear and Continuous Flow".

Trial #3 - Porcine Model Trial (in vivo) – the final trial centered on a GLP *in vivo* porcine model to assess the antibiofilm and antimicrobial activity utilizing WIS. Acute full thickness wounds were created on the back of a pig and then treated with WIS or left untreated (controls). Thereafter, a combination of bacteria (*Staphylococcus epidermidis* (coagulase-negative staphylococci (CNS))), *Pseudomonas aeruginosa* (pig clinical isolate, *Fusobacterium sp.*) were applied to these wounds to create mature biofilms and assess the infection-protective activity across the different treatments and control groups. The wounds were treated with WIS 24 hours after infection. Four hours after treatment, the dressings were removed, and all wounds were biopsied for microbiology.

Results

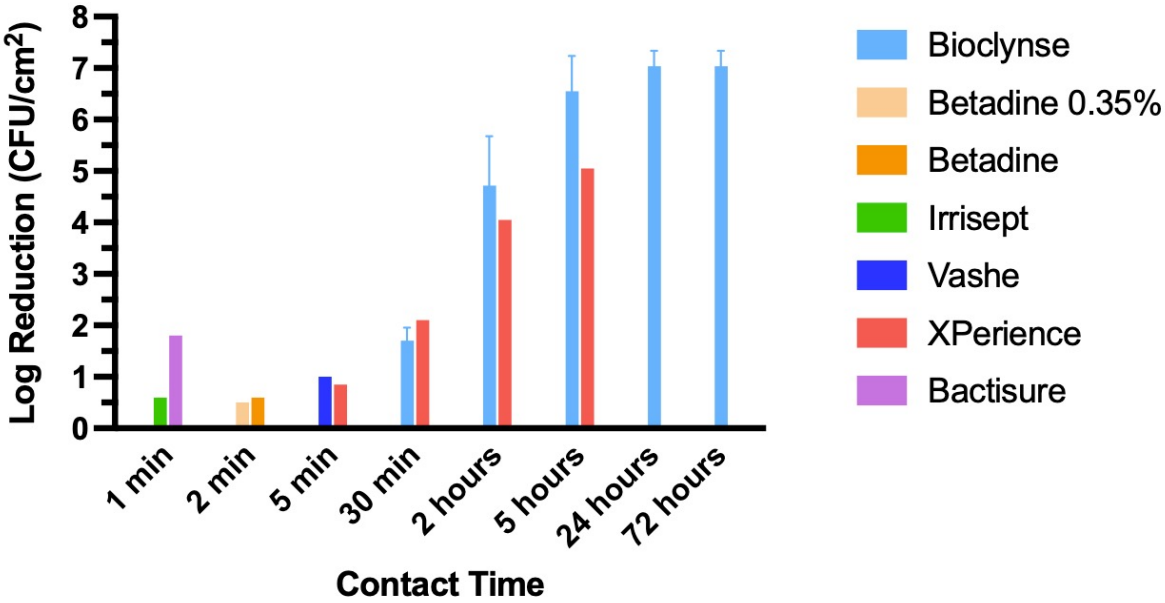
<u>Silicone Trial</u> - efficacy of WIS Copper-Iodine Complex against mature *S. epidermidis* biofilms on silicone substrate, comparison with other commercially available wound cleansers

Results: 1.7 log reduction at 30 min / 4.7 log reduction at 2 hours / 6.6 log reduction at 5 hours / 7.0 log reduction at 24h and 72h. <u>No colonies observed at 24h and 72h</u>.



Data was analyzed with one-way Anova and Tukey post-hoc tests, p-value: < 0.05

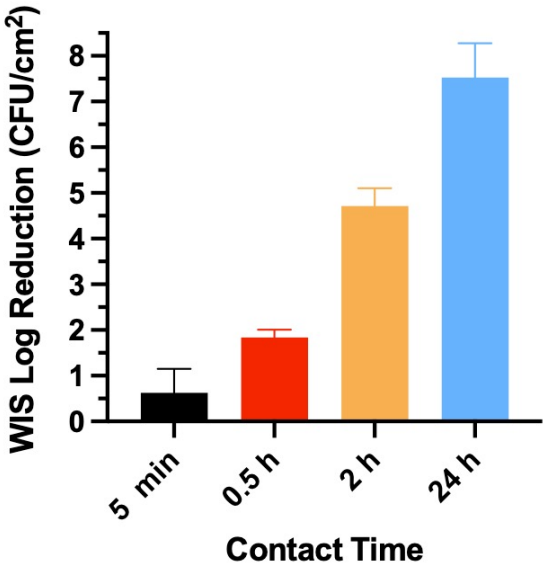
Biofilm test conducted by the Center for Biofilm Engineering at Montana State Univ.



Comparison efficacy data for other commercial wound cleansers based on published data.

<u>Titanium Alloy Trial</u> - efficacy of WIS Copper-Iodine Complex against mature *S. aureus* biofilms on titanium alloy substrate

Results: 0.6 log reduction at 5 min / 1.8 log reduction at 0.5 hours / 4.7 log reduction at 2 hours / 7.5 log reduction at 24h. <u>No colonies observed at 24h</u>.

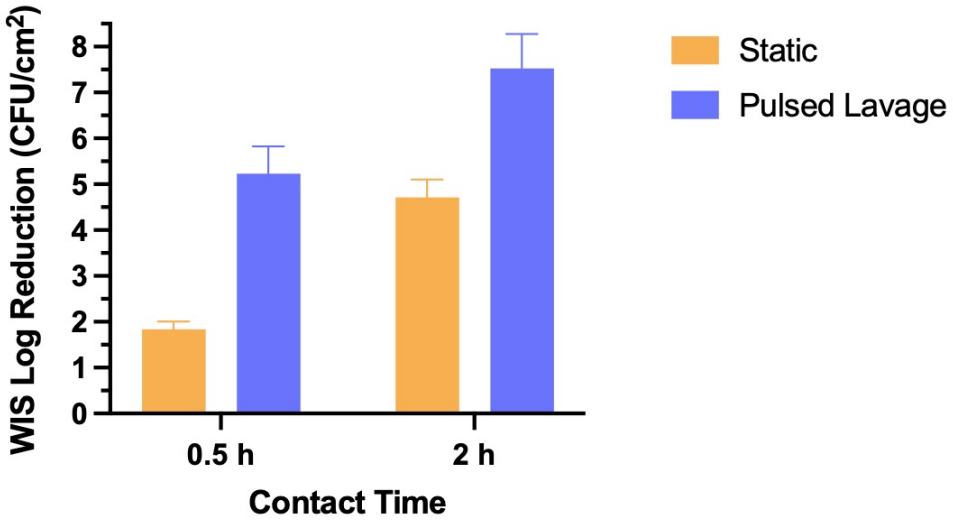


Data was analyzed with one-way Anova and Tukey post-hoc tests, p-value: < 0.05

Biofilm test conducted by the Center for Biofilm Engineering at Montana State Univ.

<u>Titanium Alloy Trial with Pulsed Irrigation</u> - further increase in efficacy of WIS Copper-Iodine Complex against mature *S. aureus* biofilms on titanium alloy substrate

Results: use of a pulsed irrigation system incorporating WIS increased the efficacy of biofilm reduction on titanium alloy substrate by up to three orders of magnitude, from 1.8 log reduction (no lavage) to 5.2 log reduction (with pulsed lavage) at 30 min, and from 4.5 log reduction (no lavage) to a full 7.5 log reduction (with pulsed lavage) at 2 hours.



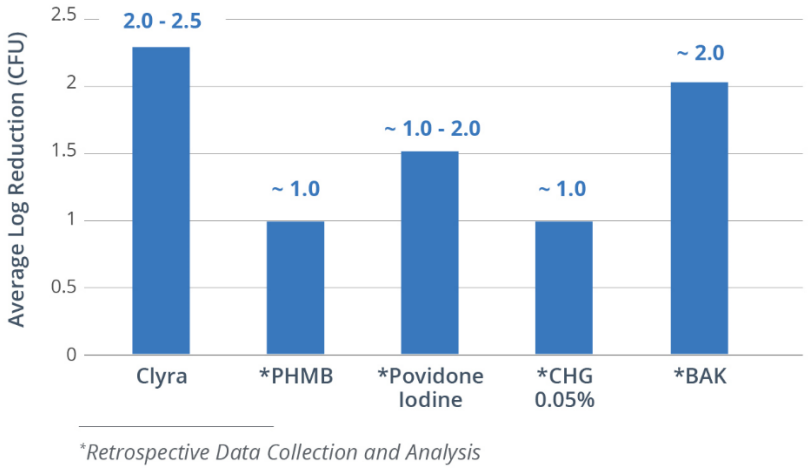
Data was analyzed with one-way Anova and Tukey post-hoc tests, p-value: < 0.05

Biofilm test conducted by the Center for Biofilm Engineering at Montana State Univ.

<u>Porcine model *in vivo* Trial</u> - to assess the anti-biofilm and antimicrobial activity.

Results: WIS Copper-Iodine Complex reduced total bacteria in the biofilm by 2.0 – 2.5 log CFUs compared to initial inoculation level.

Comparison of WIS *in vivo* antibiofilm efficacy with *in vivo* antibiofilm efficacy data of other commercially available wound cleansers drawn from published information and retrospective data collection and analysis.



*Retrospective Data Collection and Analysis

*(NOTE to Vanessa: Change "Clyra" to WIS, eliminate asterisks on 2nd to 5th labels, strike line and "*Retrospective Data Collection and Analysis beneath chart, and change axis label font to better match other charts… larger? bold?)*

Conclusion

Bioclynse® Wound Irrigation Solution generated a significant log reduction in the growth of Staph epidermidis and Staph aureus biofilms grown respectively on silicone and titanium implant materials. Biofilms were also significantly reduced in an *in vivo* wound porcine model. This offers potentially far reaching clinical applications and the chance to positively impact patient outcomes, especially considering the significant personal, clinical and financial burdens posed by septic implants. Further studies will follow to demonstrate that it can help both prevent and treat infected implants in humans.

References

1. GLP Time Kill Study 2016-2018. Nelson Laboratories
2. Antimicrobial Effectiveness Test (USP<51>) 2018. KLM Labs
3. Efficacy Test for SARS-CoV-2 Inactivation 2020. Galveston Nat'l Lab, Univ of Texas
4. Time Kill & Persistence Test 2023. Biolargo Water Laboratory, Univ. of Alberta
5. GLP Wound Healing Study (porcine wound model) 2019. Bridge PTS
6. GLP Cytotoxicity Test 2018, GLP Sensitization Test 2018, GLP Pyrogenicity Test 2018, GLP Skin Irritation Test 2019. Nelson Laboratories

- Additional statistical information and further references available on request. -